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# Giant Cell Stromal Reaction in Squamous Cell Carcinomata

## Electronmicroscopic and Ultrahistochemical Observations on the Genesis and Functional Activity of Multinucleated Giant Cells in Bleomycin-Induced Tumor Regression*

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**Summary.** Ten cases of oral squamous cell carcinoma, treated by bleomycin, were studied by electron microscopy with particular regard to the stromal reaction. The genesis and phagocytic function of multinucleated giant cells of foreign body type were observed. These cells phagocytize devitalized, keratinized tumor cells in particular. Their genesis from monocytic macrophages and endocytosis of large keratinized tumor cells are described in detail. Both phenomena are connected and the mode of formation of the cells results in functional specialization. The initial stages of intracellular digestion do not seem to take place within membrane limited vacuoles but in specialized cytoplasmatic areas which are formed around the ingested material. These contain high concentrations of hydrolases, sealed off from the rest of the cell by a clear zone of organell-free cytoplasm. This unique form of phagocytosis and digestion ("gigantophagocytosis") is only possible in these highly specialized giant cells and explains their biological significance. It is likely that secondary lysosomes are formed in subsequent stages of digestion.

The differences between our results and the experimental observations of other authors are discussed.

**Key words:** Squamous cell carcinoma – Tumor regression – Stromal reaction – Multinucleated giant cells – Macrophages – Phagocytosis – Bleomycin.

**Zusammenfassung.** 10 Fälle von Bleomycin-behandelten oralen Plattenepithelcarcinomen wurden hinsichtlich der Stromareaktion elektronenmikroskopisch untersucht. Hierbei wurde besonders die Genese und phagocytäre Funktion von mehrkernigen Riesenzellen (vom Fremdkörpertyp) beobachtet. Diese phagocytieren vor allem devitalisierte, keratinisierte Tumorzellen. Die Genese aus monocytogenen Makrophagen und die Endocytose der großen

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Keratinlamellen werden detailliert beschrieben. Beide Phänomene sind untrennbar miteinander verknüpft (funktionelle Genese) und führen zu einer funktionellen Spezialisierung. Die Initialstadien der intracellulären Digestion finden nicht in membranbegrenzten Vakuolen statt, sondern in spezialisierten Cytoplasmaarealen. Diese enthalten hohe Konzentrationen an Hydrolasen und werden durch eine organellenfreie Zone von der übrigen Zelle abgeschirmt. Diese einzigartige Form der Phagocytose und Digestion („Gigantophagocytose") ist nur in den hochspezialisierten Riesenzellen möglich und erklärt ihre biologische Bedeutung. Erst in den folgenden Schritten der Digestion kommt es zur Bildung von sekundären Lysosomen. Diese Riesenzellen besitzen eine hohe funktionelle Aktivität und Spezialisierung. Der Widerspruch dieser Beobachtung zu den experimentellen Befunden anderer Autoren wird diskutiert.

## Introduction

The occurrence of multinucleated giant cells in the stroma of malignant tumors, notably in squamous cell carcinomata has been known for a long time (Petersen, 1901; Orth, 1904; Ribbert, 1916; Konjetzny, 1918; Broders, 1920; Sirtori and Pizzetti, 1949; Hamperl, 1956; Ratzenhofer, 1970a and b). Haythorn (1929) stressed the necessity of differentiating between stromal giant cells and true tumor giant cells. The former are normally observed sporadically in the vicinity of devitalized and keratinized tumor tissue and most authors classify them as foreign body giant cells with a resorptive function. Some consider them to be "necrophages" (Ribbert, 1916), others propose a possible role in partial destruction of the tumor (Petersen, 1901; Orth, 1904; Konjetzny, 1918; Ratzenhofer, 1970a and b; cp. Evans, 1973; Adams, 1976). Ultrastructural investigations of this problem have not been reported.

New interest in this phenomenon has arisen from the observation that giant cells occur in large numbers during anti-tumor treatment with bleomycin (Renault et al., 1972; Iversen, 1974; Hornová and Bilder, 1974; Berdal et al., 1975; Burkhardt and Höltje, 1975; Burkhardt et al., 1976a). The abundance of giant cells has made a systematic investigation of this stromal reaction possible. Electronmicroscopic and enzyme-histochemical studies have demonstrated unequivocally that the giant cells are not tumor giant cells but macrophage polykaryons formed by fusion of macrophages of monocytic origin (Burkhardt et al., 1976a).

The present study provides a more detailed description of the significance and mechanism of the genesis and of the phagocytic activity of these giant cells.

## Material and Methods

This study is based on observations in 10 cases of oral squamous cell carcinomata treated by intra-arterial bleomycin therapy, followed by biopsy or excision of the tumor (total bleomycin dose ranging from 225 mg to 510 mg; cp. Höltje et al., 1976; Burkhardt et al., 1976b).

The tissue was processed for electron microscopy according to the method specified by Luft (1971a, b). After dehydration in a graded series of alcohols, embedding in Epon 812 was carried out. In semithin sections, stained by toluidine blue, appropriate areas for electron microscopy were selected. Ultrathin sections were contrasted with alcoholic uranylacetate. Analysis was done with the Zeiss Elektronenmikroskop Em 9 S-2. Ultrahistochemical demonstration of acid phosphatase activity was performed by the method of Komiyama and Spicer (1974) modified by Pearse (1968). $\beta$-glycerophosphate was used as substrate. The reaction was done at a pH of 5.8. Acid phosphatase activity was indicated by precipitates of lead-phosphate, which is formed as the result of hydrolysis of $\beta$-glycerophosphate in the presence of lead-ions (lead acetate).

## Results

Macrophages and giant cells could be demonstrated in the tumor area in all cases, arranged in granulomas in some instances. Different stages of maturation of the macrophagocytic cells could be observed. There were immature cells corresponding to blood monocytes and transitional stages to mature macrophages and giant cell formation (Fig. 1). The latter cell types exhibited avid phagocytic activity.

The mononuclear macrophages and to a lesser degree the giant cells formed cytoplasmatic projections and filopodia, which were pushed between the keratinized tumor cells (Fig. 1). Here they broke down the connections of the keratinized squames (modified desmosomes and dovetailing), which are of varying strength depending on the degree of tumor differentiation. Three dimensionally, and functionally, the cytoplasmatic projections must be viewed as undulating membranes, veil like membranes and flap like expansions, as shown in scanning electron microscopic observations (Warfel and Elberg, 1970; Papadimitriou et al., 1973a; Parakkal et al., 1974). They were apparently actively breaking up the massive keratinized tumor area into single keratin lamellae.

From the size of these lamellae endocytosis by mononuclear macrophages seemed to be impossible. A number of mononuclear macrophages, or more often mononuclear macrophages in cooperation with established giant cells, engulfed the keratinized tumor cells (Figs. 1 and 2). This process was accompanied by deep interdigitations of the cellular membranes of the phagocytic cells, which interlocked in zipper-like arrays (Fig. 2). Acid phosphatase activity could be demonstrated along the interdigitations (Burkhardt et al., 1976a). Through fusion of the cellular membranes along these interdigitations giant cells were formed or enlarged and the keratinized cells were ingested (Fig. 2). The nuclei of the giant cells were arranged in a nuclear concentration area usually distant from these endocytic enclosures (Fig. 2a). In early stages the largely unaltered keratinized cells were seen inside the peripheral cytoplasm of the giant cells, conspicious by the well demarcated horny cell membranes. Internally they were composed of fibrillar keratin, residues of the nucleus and organelles as well as lipid vacuoles (Fig. 3a). Acid phosphatase activity could be demonstrated around these still "juicy" keratinized cells (Fig. 3b) and "digestive areas" were formed.

It is noteworthy that often no limiting membrane could be seen in these areas, i.e. a digestive vacuole was not formed. Instead, a special differentiation

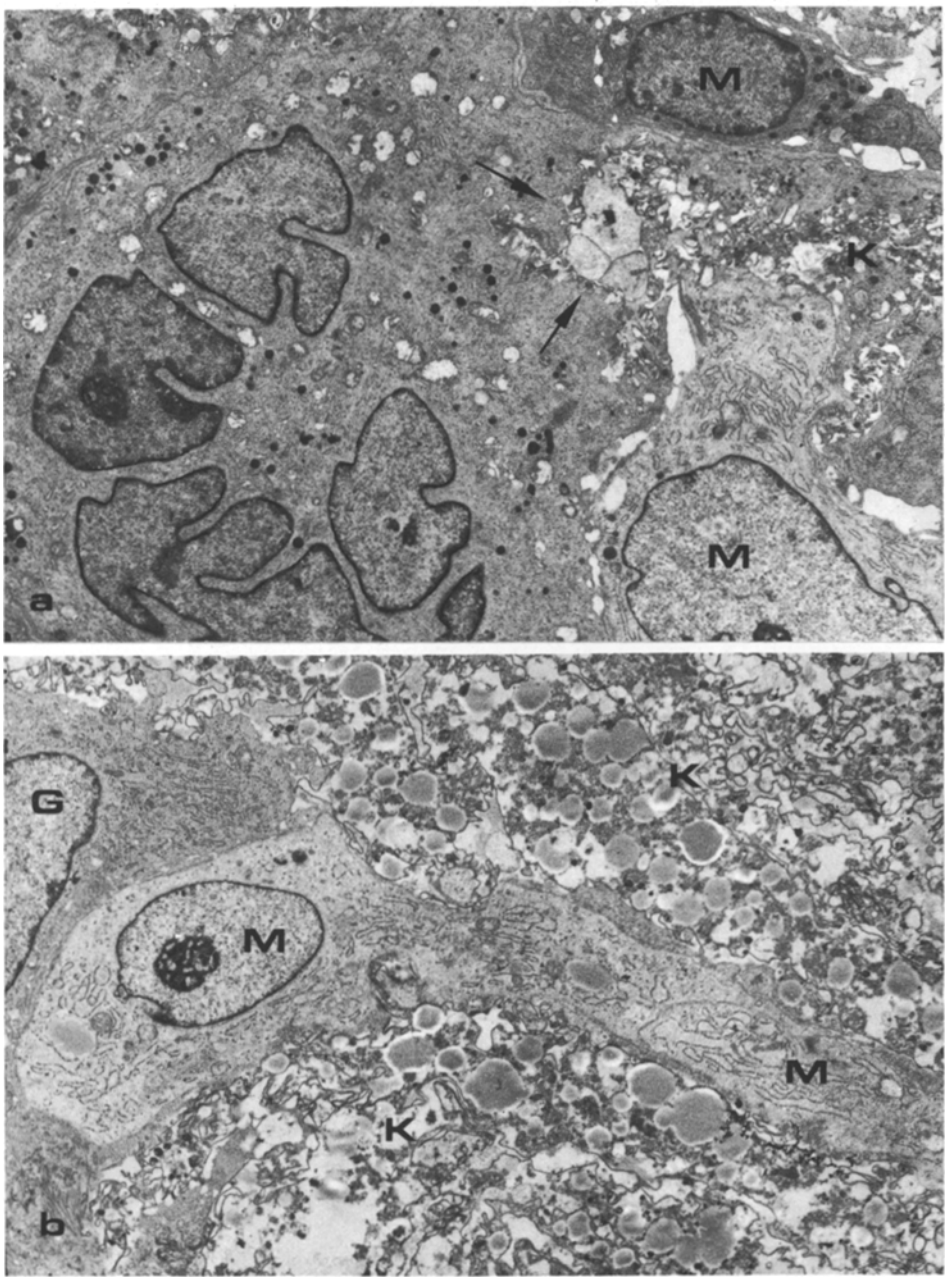


**Fig. 1a and b.** Development of macrophages and giant cells at the site of resorption of keratinized tumor cells (*K*). **a** Engulfment of keratinized cells by a small giant cell with numerous small lysosomes in close association with two differently matured macrophages (*M*). Part of the keratin squame has already been ingested (*arrows*). ×3800. **b** Immature macrophage in association with a giant cell (*G*) and a further mononuclear macrophage (*M*) breaking up the keratinized tumor (*K*) by the formation of a long cytoplasmatic process. The keratinized tumor cells show conspicious cell membranes and numerous lipid vacuoles. ×5100

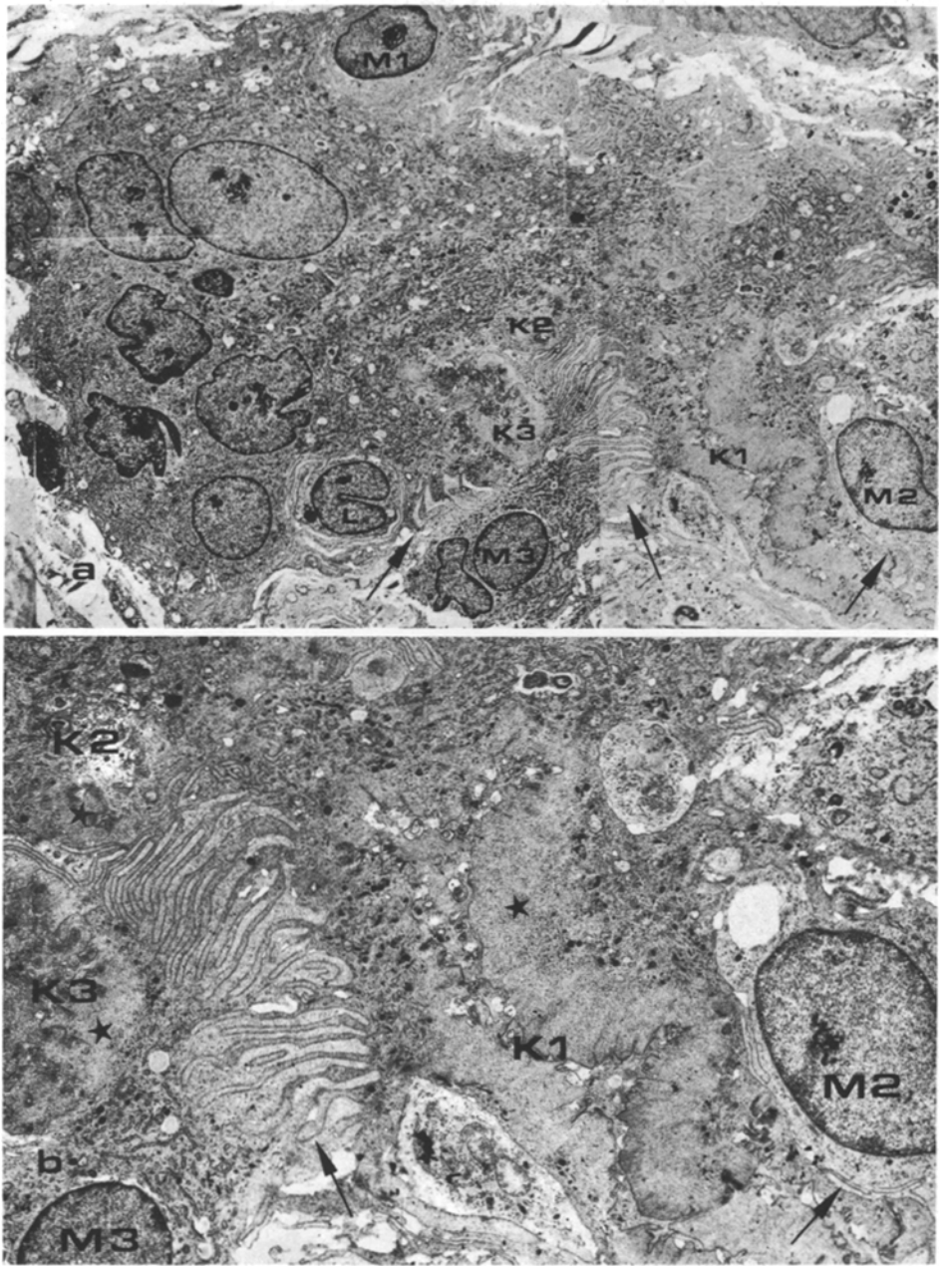


**Fig. 2a and b.** Mature, actively phagocytizing giant cell with ingested keratinized tumor cells (*K1, K2, K3*). The nuclei are concentrated in an area remote from the phagocytic inclusions. The giant cell is associated with three phagocytic cells (*M1, M2, M3*) and a lymphocyte (*L*). The mononuclear macrophage *M2* and the binucleated giant cell *M3* are directly related to the ingestion of the keratinized cells. They interlock in zipper-like arrays by deep and closely apposed interdigitations (*arrows*) of the cellular membranes thereby enclosing the keratinized cells through fusion with the giant cell. Note the clear zone surrounding the ingested keratinized cells (*asterisk*). *K1* is in an early stage of digestion. The outer membrane of the horny cell is still conspicious, a limiting membrane by the giant cell can be seen in some parts. *K2* and *K3* are in an advanced stage of digestion, a surrounding membrane of the giant cell, i.e. phagocytic vacuole, cannot be demonstrated. **a** Survey. ×2100. **b** Detail of a. ×4500

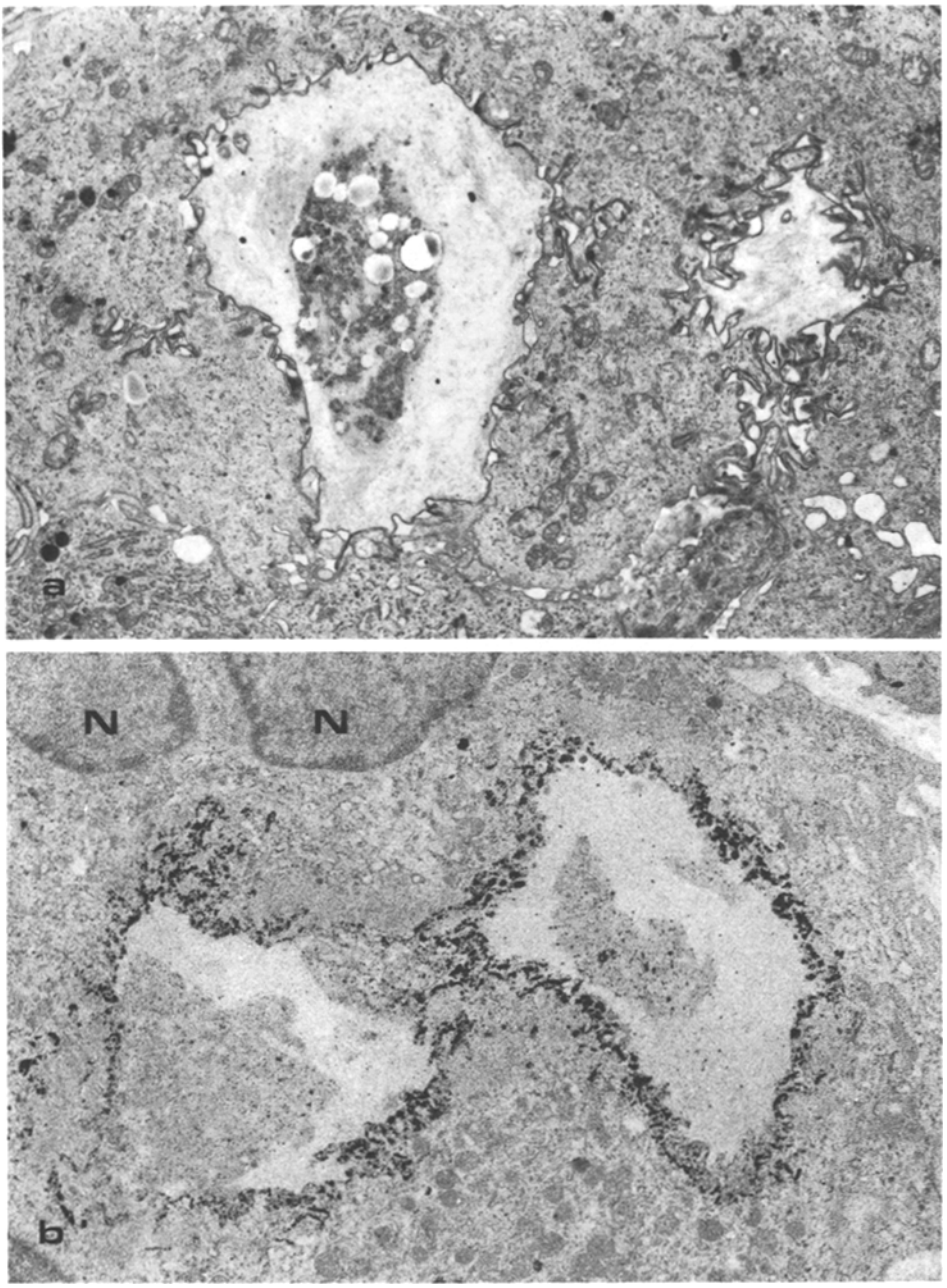


**Fig. 3a and b.** Early stage of digestion of keratinized tumor cells. **a** A keratinized cell with well preserved fibrillar keratin, lipid vacuoles, remnants of organelles and nucleus and a well demarkated outer membrane showing initial infolding is enclosed by a giant cell. A surrounding membrane of the giant cell can be demonstrated in most parts, a clear zone has not yet been completely established. ×6100. **b** Demonstration of acid phosphatase activity in the vicinity of ingested keratinized cells. *N* Nuclei of the giant cell. ×6800

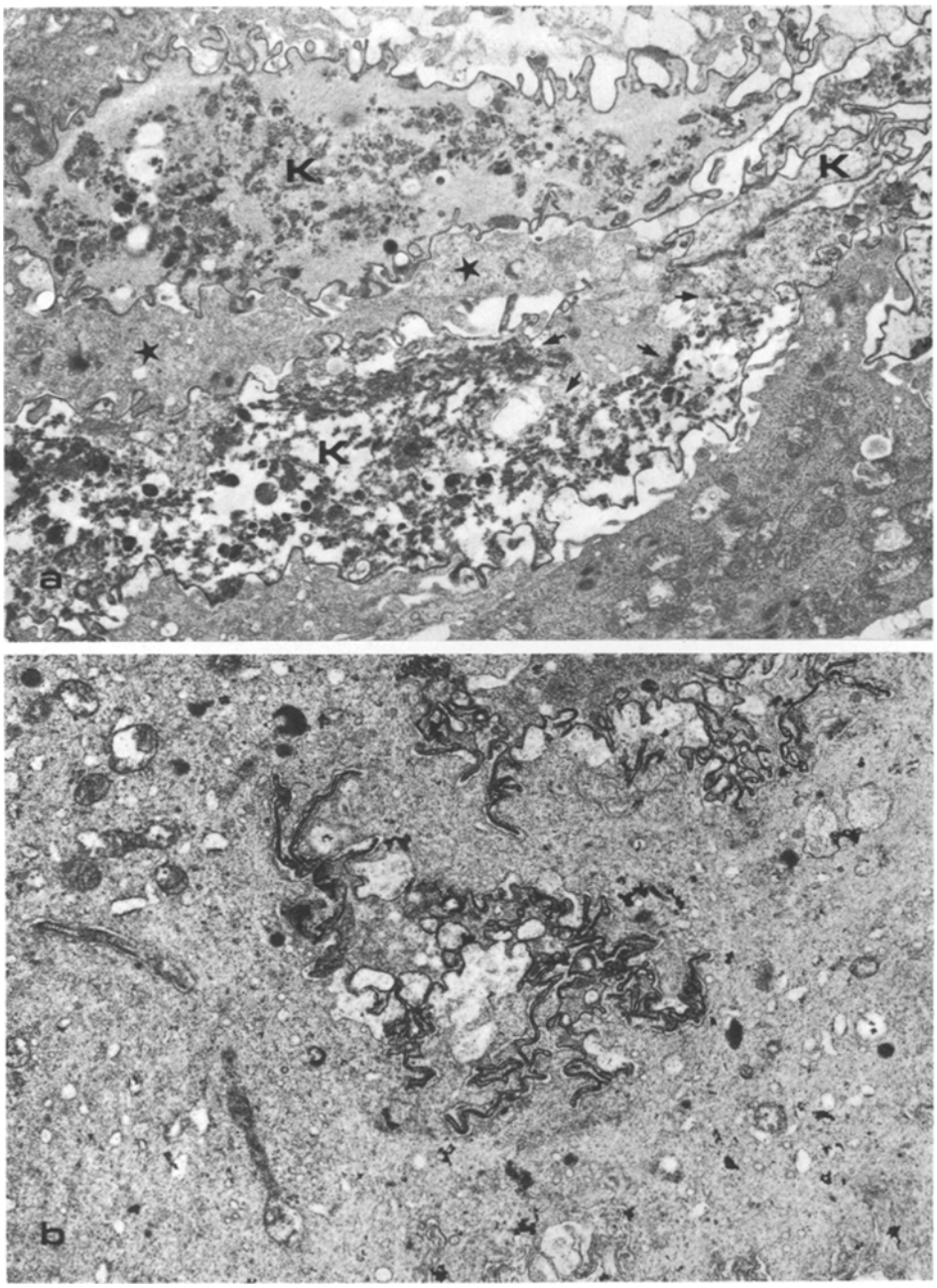


**Fig. 4a and b.** Breaking up of the outer membrane of the keratinized tumor cells and resorption of the keratin contents. **a** Several ingested keratinized cells (*K*). Between two of these a clear zone of cytoplasm (*asterisk*) has been established (no limiting membrane), which at one point has broken up the enveloping membrane of the keratinized cell (*arrows*). The keratin shows granular degradation. The cytoplasm of the giant cell in the lower part of the picture still has a partly demonstrable cytoplasmic membrane and contains organelles. ×6900. **b** Collapsed keratinized cell with only little keratin contents and apposed outer membranes in the periphery. These are folded. Surrounding cytoplasm shows paucity of organelles, only a few lysosomes are seen. ×9700

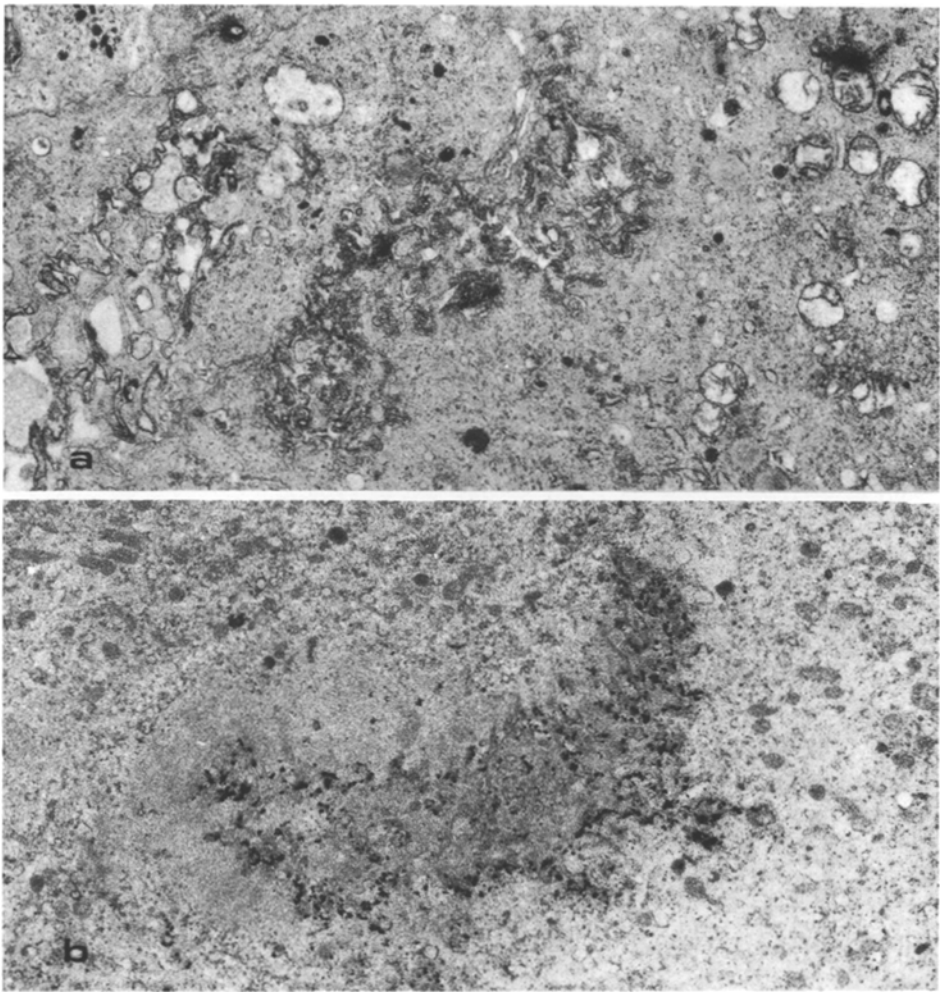


**Fig. 5a and b.** Advanced stages of digestion of the keratinized tumor cells. **a** Empty outer membrane of a keratinized cell with dissolution in some parts. No digestive vacuole is demonstrable. Note clear zone in the vicinity. ×8100. **b** Demonstration of acid phosphatase at the inner rim of the clear zone. ×6100

of the cytoplasm surrounding the ingested material was observed, consisting of a clear, finely granular, homogeneous zone free of organelles (Figs. 2, 4, 5, 6). High phosphatase activity could be demonstrated in the clear zone. It did not show a membrane limited distribution (Figs. 3b, 5b). Apparently the resistant outer membrane of the keratinized cell is a barrier to digestion. Small parts of the membranes were broken down and cytoplasm of the phagocytic cell with hydrolytic enzymes was apparently pushed into the interior (Fig. 4a). Degradation and resorption of the keratin follows. However, most of the outer membrane of the keratinized cells remained intact and collapsed in bizarre

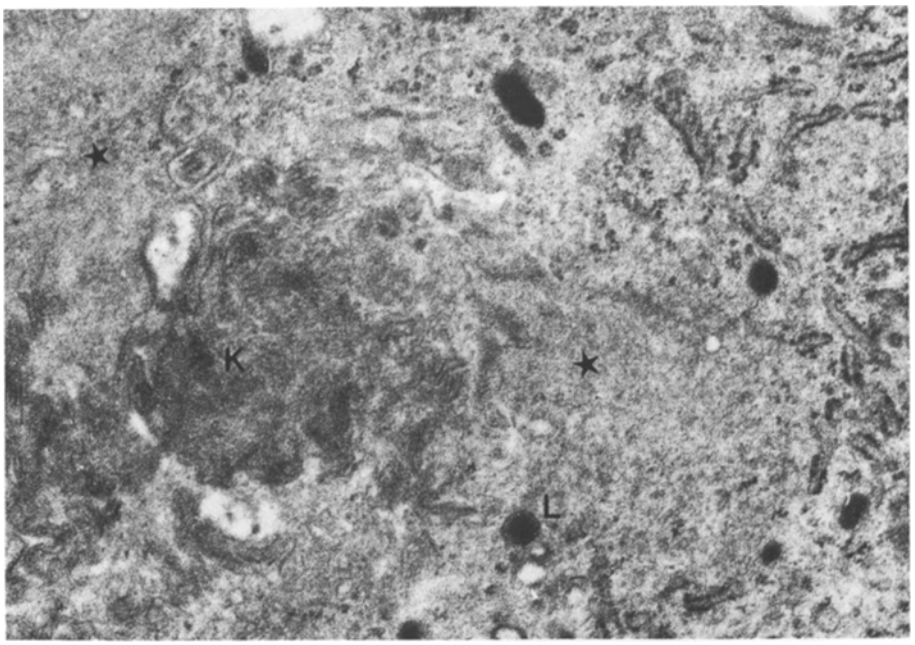


**Fig. 6.** High magnification of the ingested keratinized cell (*K*) in an advanced stage of dissolution. A limiting membrane of the giant cell is not demonstrable. *Asterisk* = clear zone, *L* = primary lysosome. At the right well delineated profiles of the rough endoplasmatic reticulum. ×31,000

figures (Figs. 4, 5). These were folded and rolled up, reducing their volume. Focal dissolution could be observed (Figs. 5a, 6). The stable membrane proteins liberated could be the source of the abundant myelin-like whorls in the cytoplasm of the giant cells (Fig. 7). The latter were then further degraded, lysosomes encircled them (Fig. 7b) and complex phagolysosomes with demonstrable acid phosphatase activity were formed (Fig. 8). In the larger and presumably older giant cells with an increased number of nuclei, wide areas of the cytoplasm were often filled with these phagolysosomes (Fig. 8a).

## Discussion

During treatment of squamous cell carcinomata with bleomycin a maturation (prosoplasia) of the tumor occurs. The tumor cells are devitalized by keratinization, while simple necrosis plays a minor role (Burkhardt et al., 1976a). The minimal myelo- and immunosuppressive action of bleomycin permits a marked granulomatous inflammation, including abundant multinucleated giant cells, to surround the tumor. This seemed a suitable model to provide information on stromal reactions in carcinomata and the biological behaviour of giant cells.

Although there have been numerous investigations on the formation, enzyme histochemistry and ultrastructure of foreign body giant cells in granulomatous

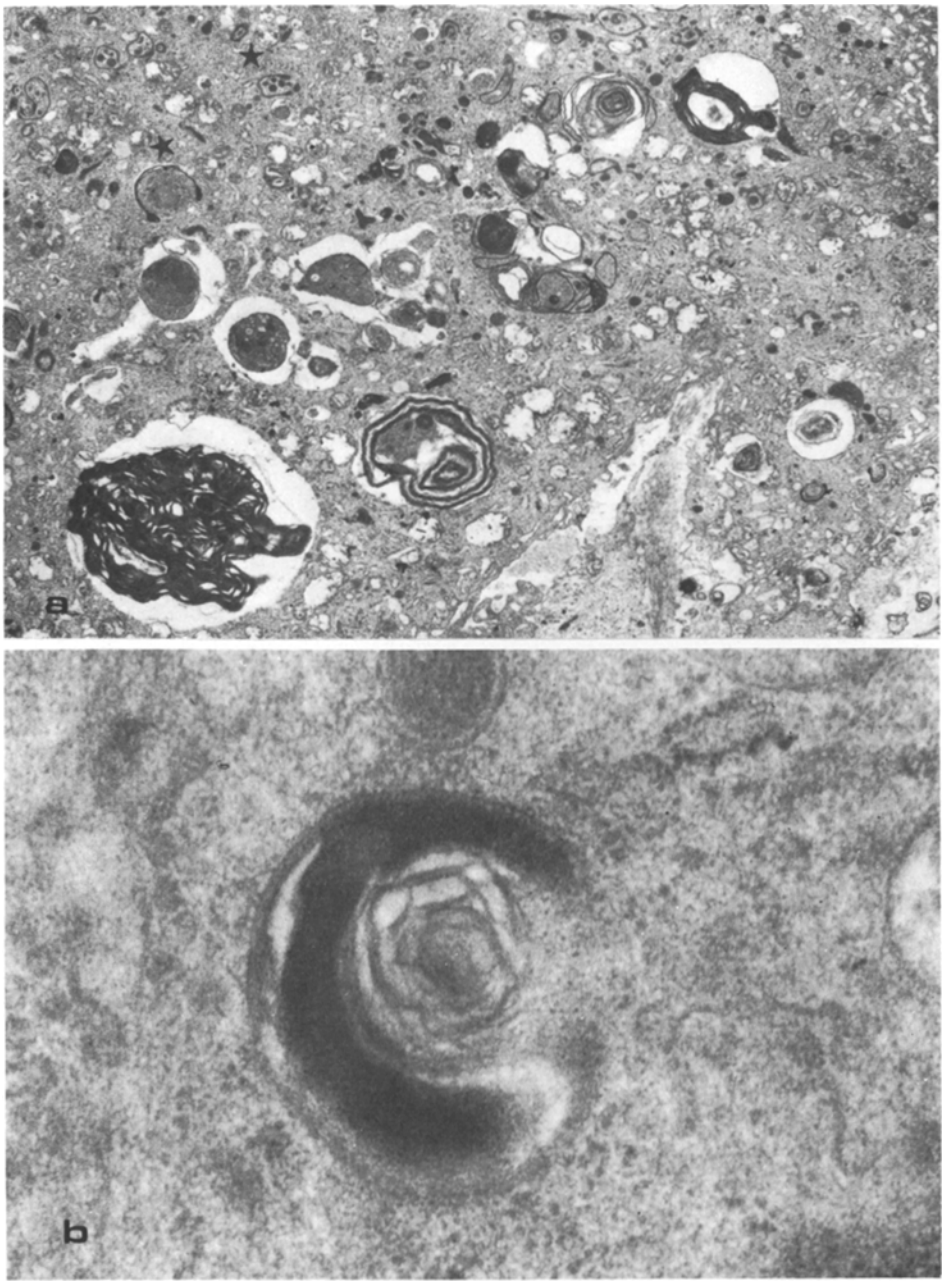


**Fig. 7a and b.** Formation of myelin bodies and secondary lysosomes. **a** Numerous and variously shaped myelin bodies in the peripheral cytoplasm of a giant cell. Fusion with lysosomes can be observed (*asterisk*). × 5300. **b** Myelin body encircled by a lysosome. Development of a secondary lysosome. × 70,000

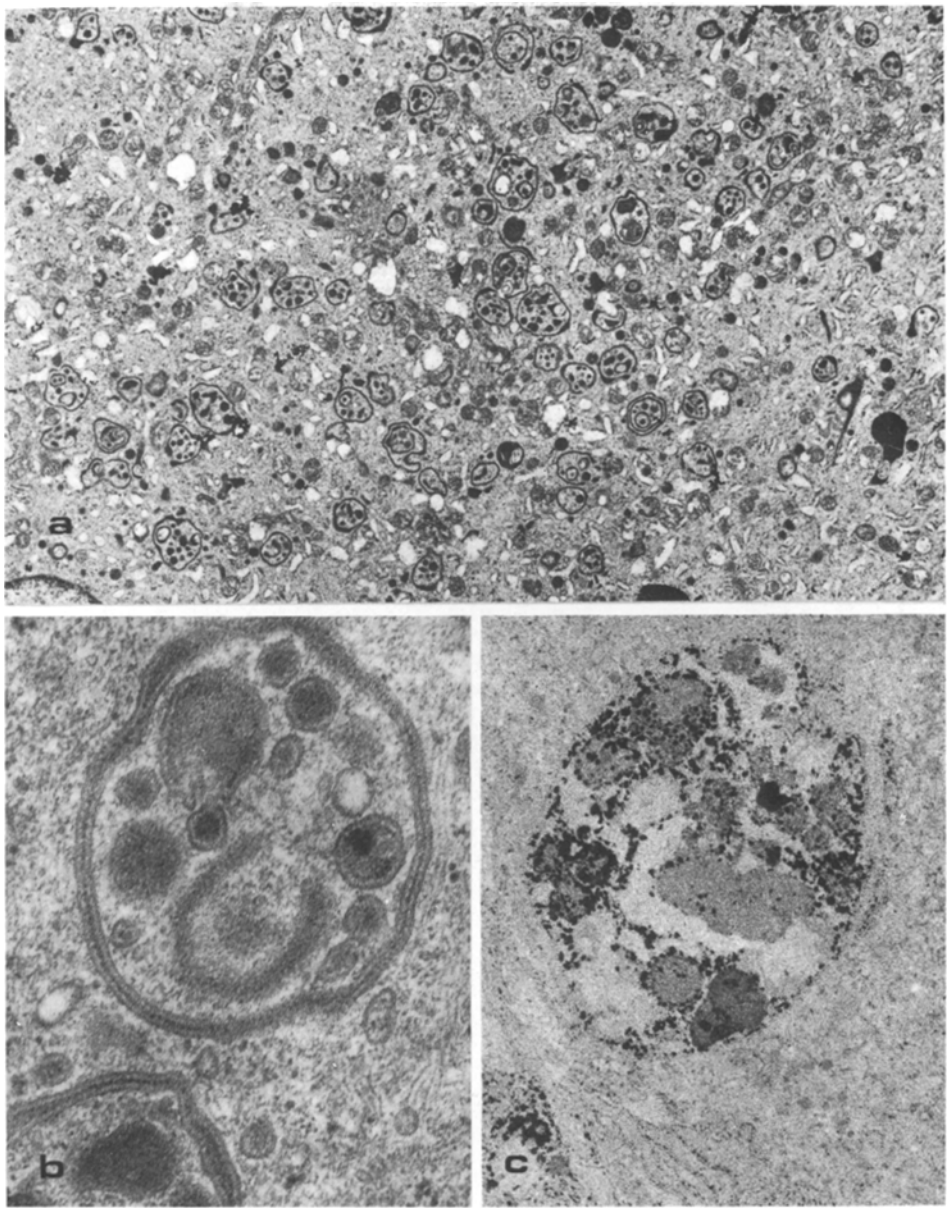


**Fig. 8a–c.** Final stage of digestion with formation of phagolysosomes. **a** Myriads of variously shaped phagolysosomes in the peripheral cytoplasm of a giant cell. ×5300. **b** Higher magnification of a complex phagolysosome. ×48,000. **c** Demonstration of acid phosphatase in a phagolysosome. ×48,000

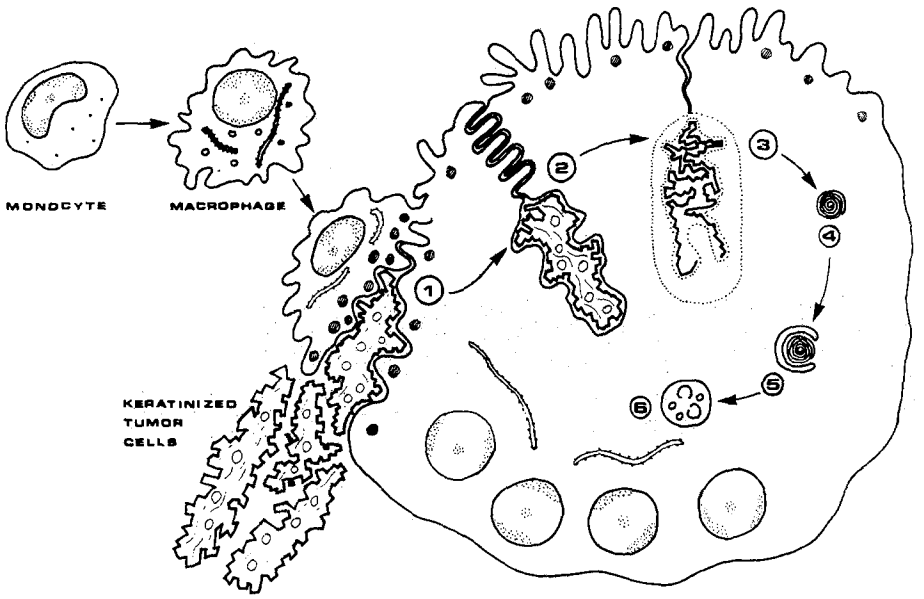


**Fig. 9.** Schematic synopsis of the functional genesis and phagocytic activity of the multinucleated giant cell. 1. Site of phagocytosis of giant cell and associated macrophage. Simultaneous ingestion of the keratinized tumor cells and interdigitation/fusion of the membranes of the phagocytic cells. 2. Engulfed tumor cell still surrounded by a membrane. 3. Establishment of a digestive area with a clear zone (indicated by the dotted line). The enveloping membrane of the horny cells is partly melted down, the keratin is absorbed and the membranes collapse. 4. Residual membrane proteins form myelin-bodies. 5. The myelin-bodies are taken up by lysosomes. 6. Formation of complex phagolysosomes

inflammation, both under experimental conditions and in cell culture, their significance and function in human pathology remains obscure. Sutton (1967) has stated "the giant cell remains an enigma". There is now general agreement that they are formed by fusion of monocytogenic macrophages (Lambert, 1912; Lewis, 1927; Haythorn, 1929; Leder and Nicolas, 1965; Gillman and Wright, 1966; Sutton, 1967; Carter et al., 1970; Carter and Roberts, 1971; Papadimitriou et al., 1973b; Adams, 1974, 1976; Black and Epstein, 1974; Mariano and Spector, 1974; Papadimitriou and Archer, 1974; Papadimitriou and Wyche, 1974; Black et al., 1976; Sapp, 1976), and not by amitotic nuclear division (cp. Wurm, 1956; Gusek, 1958; Bassermann, 1961; Leder and Nicolas, 1965; Hodel, 1967).

A similar origin and mode of formation could be demonstrated for giant cells in the stroma of bleomycin-treated carcinomata (Burkhardt et al., 1976a). While the origin and genesis of the multinucleated giant cells is largely agreed, their function, phagocytotic ability, and activity is still a matter of controversy. There is a striking discrepancy between the results of different investigations. The enzymatic pattern (Gössner, 1956; Gedigk and Bontke, 1957; Hodel, 1967; Papadimitriou and Wyche, 1974), cytophotometric observations (Queisser et al., 1968) and ultrastructural features of giant cells (Gusek, 1955, 1958, 1964; Basser-

mann, 1961; Bönicke et al., 1963; Wanstrup and Christensen, 1966; Sutton, 1967; Papadimitriou and Archer, 1974; Mariano et al., 1976) favour an intensive and wide range of metabolic activities with marked versatility. On the contrary the actual phagocytic activity observed under experimental conditions, in cell culture or in inflammatory granulomas has been found to be minimal (Bassermann, 1961; Sutton, 1967; Mariano and Spector, 1974). In these experiments giant cells are usually produced in culture, by implanting glass, plastic, or talcum particles subcutaneously or by injecting various substances in animals (mostly mice) or humans. Phagocytic activity is investigated by offering them bacteria (Mariano and Spector, 1974; Papadimitriou et al., 1975), altered erythrocytes (Papadimitriou et al., 1975), coal particles (Carter et al., 1970) or colloidal particles (Papadimitriou and Archer, 1974). Under these conditions giant cells exhibit a poor phagocytic ability compared to mononuclear macrophages (Sutton, 1967; Carter et al., 1970; Carter and Roberts, 1971; Mariano and Spector, 1974; Papadimitriou and Archer, 1974). A decrease of phagocytic performance with increase in the number of nuclei was observed by Papadimitriou et al. (1975).

This discrepancy between the potential and actual functional abilities of giant cells has led to different interpretations of the function and significance of the cells. Some authors simply consider them as defunct, debilitated cells representing ageing of macrophages. Papadimitriou (1973) and Papadimitriou et al. (1975) demonstrated paucity of receptor sites for the Fc portion of heterologous IgG and discuss a loss of some surface receptors subsequent to fusion. Mariano and Spector (1974) suggest that giant cell formation is a disposal mechanism for unwanted macrophages in inflammatory reactions with low toxicity, and a surplus of macrophages.

On the contrary Black and Epstein (1974) consider giant cell formation to be a reaction to a toxic environment.

In contrast to all of these observations we have demonstrated avid phagocytic activity of multinucleated giant cells in bleomycin-treated carcinomata. The different stages of tumor destruction, keratinization, and resorption permit a reconstruction and functional interpretation of the macrophagocytic process. A schematic presentation of this is given in Fig. 8. The cooperation of macrophages and giant cells in phagocytosis is determined by two factors: The size of the ingested keratinized squames and their highly resistant outer membranes. These require a special mode of ingestion and digestion. Our observations show a successive addition of macrophages to an initial cell during the phagocytic process rather than a simultaneous fusion of a number of cells (cp. Carter et al., 1970; Mariano and Spector, 1974). Cell membrane fusion is achieved through membrane projections and interdigitation of the membranes. This is a general mode of membrane fusion (Davis, 1963; Sutton, 1967) reducing electrostatic repulsion (Poste and Allison, 1971). The membrane fusion reaction requires in addition an alteration of the cell coat, which can be induced by lysosomal enzymes (Poste and Allison, 1971). Acid phosphatase activity could be demonstrated along these interdigitations in foreign body giant cells (Papadimitriou and Archer, 1974) as well as in giant cell formation during bleomycin treatment of carcinomata (Burkhardt et al., 1976a).

Thus the formation of giant cells is a process directly connected with the ingestion of the "foreign particle", the zipper-like closure (interdigitation) of the cell membranes, formation of digestive areas, discharge of hydrolytic enzymes and the consequent membrane fusion. We consider this to be not only the mode of formation of these cells but also a process producing macrophages with effective functional specialization at their site of action. This conception is in line with the observations of Adams (1974, 1976) that "the characteristic features of granulomatous inflammation are a result of differentiation and that maturation lies in direct relation to function".

The process of fusion is followed by assembling the specialised regions of the cells into common zones and formation of a cytocentrum (Lewis, 1927; Papadimitriou and Archer, 1974).

The discharge of lysosome-contents into the digestive area during ingestion can apparently be very rapid. This would explain the fact that some giant cells are almost depleted of lysosomes, simulating inactivity. Sutton and Weiss (1966) observed a loss of lysosomes in ageing giant cells and explained this fact by discharge into phagocytic vacuoles and into the external environment. In agreement with this we could demonstrate high concentrations of acid phosphatase in the digestive areas. Other cells contained numerous small newly formed primary lysosomes.

Our observations seem to indicate that the ingestion of the large keratinized tumor cells into the cytoplasm of the giant cell is followed by an unusual mode of intracellular digestion. Primarily there is no distinct digestive vacuole or formation of phagolysosomes, as in mononuclear phagocytosis (Dingle, 1968; Daems et al., 1969; Langer, 1976). The enveloping plasma membrane of the giant cell is apparently melted down partly or completely and high concentrations of hydrolases are built up around the keratinized tumor cells. The hydrolases are not membrane limited at this stage. This supports our suggestion that a not-membrane-bound step of digestion exists. The surrounding cytoplasm of the giant cell shows a fine granular area which is free of organelles. This corresponds to the clear zone or hyaline rim of osteoclasts (Holtrop et al., 1974; Schenk, 1974). By analogy we interpret this zone as a seal protecting the rest of the cell against autodigestion by the highly concentrated hydrolases. This hypothesis provides another explanation of the biological significance of giant cell formation: Only a voluminous cell body can afford to build up such a high internal hydrolytic activity and seal this off from the rest of the cell. A smaller mononucleated cell would be damaged by the enzymes. A regurgitation of hydrolases into the immediate surroundings would result in tissue injury as when neutrophil leucocytes are exposed to immuncomplexes distributed along a non-phagocytosable surface (Henson, 1971). Papadimitriou and Wee (1976) showed that giant cells are capable of selective enzyme exocytosis under experimental conditions following plastic implantation – this must be considered as an unnatural condition (see below). The formation of large vacuoles in cells is in addition limited by physical factors such as surface tension.

This mode of digestion can be considered as a combination of extracellular and membrane-bound intracellular digestion (cp. Dingle, 1968; Langer, 1976) and might be termed "gigantophagocytosis". It shows many parallels to the

highly specialized activities of osteoclasts (cp. Sutton, 1967) and highly active osteoclasts in Paget's disease can exhibit a similar dissection and ingestion of large bone fragments (Schulz, 1977). This specialized ability might be a common feature of different kinds of giant cells, exhibiting high acid phosphatase and phagocytic activity. The enveloping membrane of the keratinized tumor cells is relatively resistant to digestion. It is a specialized plasma membrane of keratinocytes, possibly formed by membrane coating granules during keratinization. It consists of a highly stabilized amorphous substance residing in the thickened inner part of the horny cell membrane, functioning as an exoskeleton (Kligman, 1964; Nicolaides, 1964; Rowden, 1966; Matoltsy and Parakkal, 1965; Matoltsy et al., 1974; Breathnach, 1975). This membrane also has a higher resistance to chemical degradation than keratin and functions as a protective barrier in the epidermis (Matoltsy and Balsamo, 1955).

It seems that the giant cell therefore digests the keratin first and empty membranes linger for a longer time in the cytoplasm still surrounded by hydrolases. Finally these are broken down and remnants of the structural proteins might form the observed myelin bodies (cp. Wanstrup and Christensen, 1966; Otto, 1970). These could be further degraded by the formation of phagolysosomes and residual bodies. Emiocytosis has not been observed.

From our observations it is not possible to draw a conclusion on the fate of the giant cells (emigration, dissociation, death). From experimental data it is known that the lifespan of the cells is limited (Gillman and Wright, 1966: 2–3 weeks; Papadimitriou et al., 1973b: half life span of few days; Mariano and Spector, 1974: up to 6 days), but their ultimate fate is not certain (Papadimitriou et al., 1973b; Adams, 1976).

These observations show that the multinucleated giant cell is a highly specialized, phagocytic cell when observed under natural conditions. Experimental results indicating a poor phagocytic activity may be explained by the unnatural environment and the artificial conditions used to promote giant cell formation. Plastic and glass implants are unphagocytosable even for giant cells, so such reactions might be considered atypical from the start. It is known that environmental stimuli are essential for an adaptive response of digestive enzyme levels (Cohn and Benson, 1965).

A further important variable in these experiments is the fact that the formation of giant cells is induced by a different agent than the material which is used to test phagocytic activity. According to our observations these two phenomena are indivisibly combined.

In conclusion, resorptive activity in the stromal reaction of squamous cell carcinomata is a morphologically and functionally complex process of cooperation of macrophages. This, again, is only a part of the stromal reaction during tumor regression, which is further characterized by immunological cellular interactions and cytotoxic activity of macrophages and lymphocytes, appearance of stimulated plasma cells, concomitant nonspecific inflammatory infiltrate and changes in the connective tissue.

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